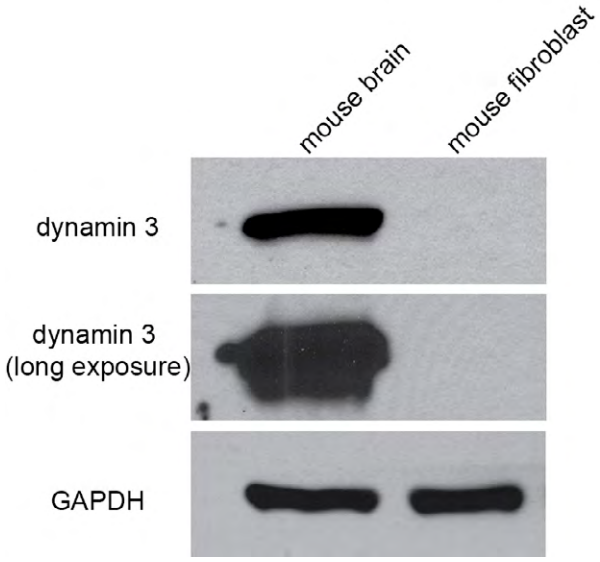


Fig. S1. Very low expression levels of dynamin 3 in cultured mouse fibroblasts relative to its expression in mouse brain. Lysates from wild-type mouse brain and fibroblasts were immunoblotted with an anti-dynamin 3 antibody. GAPDH is included as a loading control. Dynamin 3 is undetectable in the fibroblast sample, although it is detectable by mass spectrometry in these cells (see text).

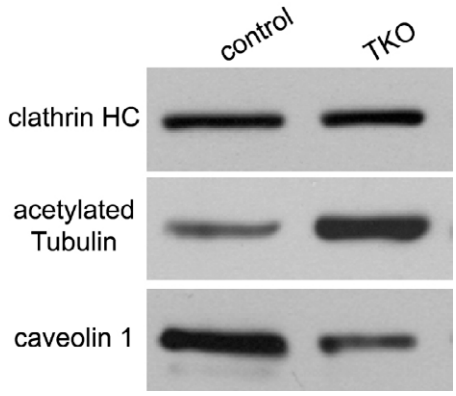


Fig. S2. Altered caveolin and acetylated-tubulin levels in dynamin TKO cells. Representative immunoblots of total cell homogenates from control and dynamin TKO cells. The anti-clathrin HC blot is included as a loading control.

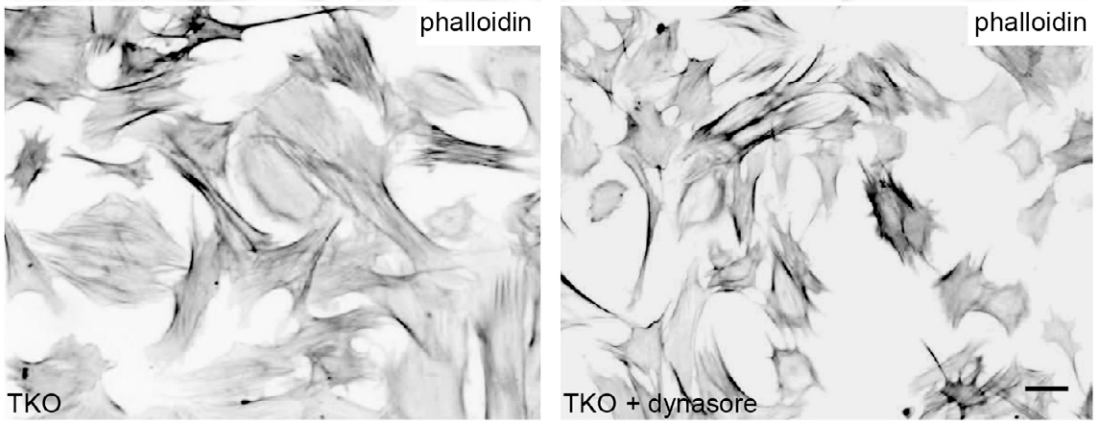


Fig. S3. Dynasore affects cell morphology. Fluorescent (AF594) phalloidin staining of fixed TKO cells incubated for 30 min at 37°C in the absence (left) or presence (right) of dynasore demonstrates dynasore-induced cell shrinkage. Scale bar=50 μm.

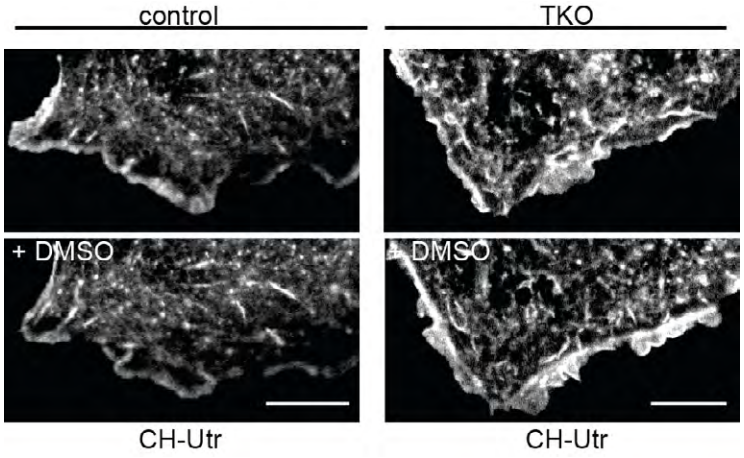
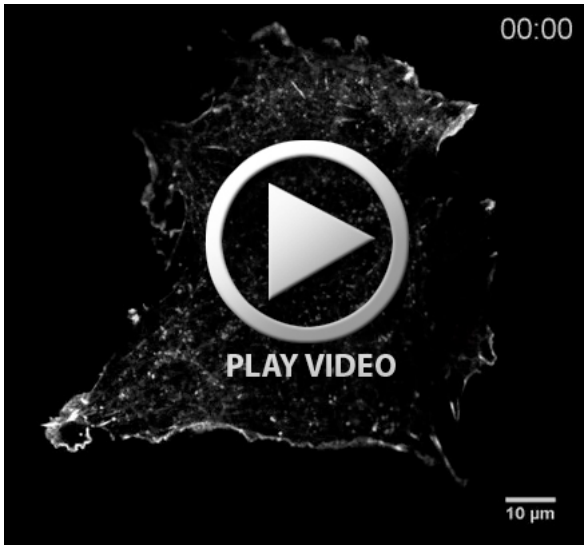


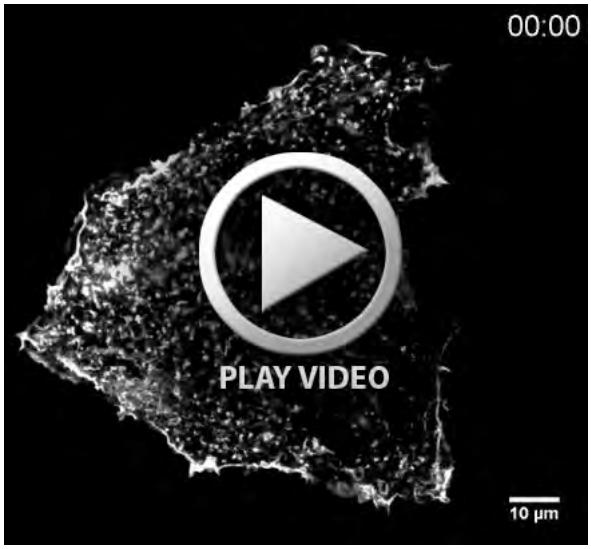
Fig. S4. The concentration of DMSO present in the medium of dynasore-treated cells (DMSO is needed to solubilize dynasore) does not affect peripheral membrane ruffling in wild-type and dynamin TKO fibroblasts. Fluorescence images of small regions of the cell periphery of a control (left) and a TKO cell (right) expressing CH-Utr fused to GFP. Cells were imaged before and after 20 min incubation with 0.15% DMSO by spinning disk confocal microscopy. Scale bar=10 μm.



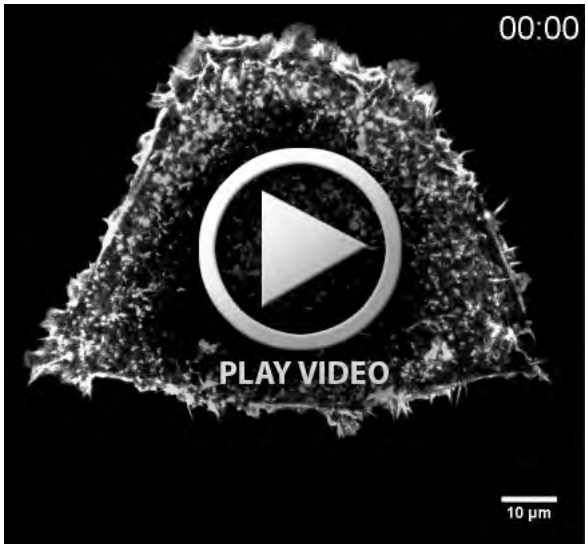
Movie 1. Control cell expressing CH-Utr-GFP treated with DMSO

Movie 2. Control cell expressing CH-Utr-GFP treated with dynasore

Movie 3. Control cell expressing CH-Utr-GFP treated with Dyngo-4a

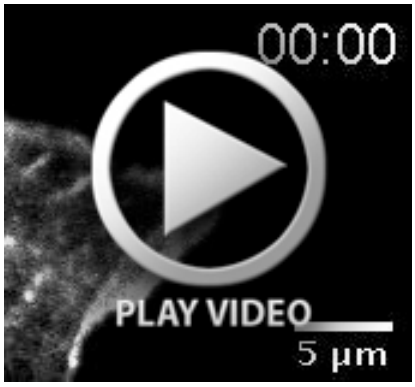


Movie 4. Dynamin TKO cell expressing CH-Utr-GFP treated with DMSO



Movie 5. Dynamin TKO cell expressing CH-Utr-GFP treated with dynasore

Movie 6. Dynamin TKO cell expressing CH-Utr-GFP treated with Dyngo-4a

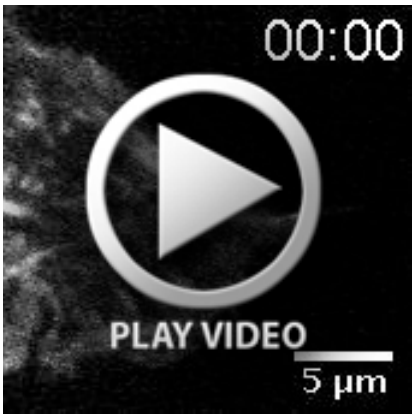


Movie 7. Cropped region of a control cell expressing CH-Utr-GFP treated with DMSO

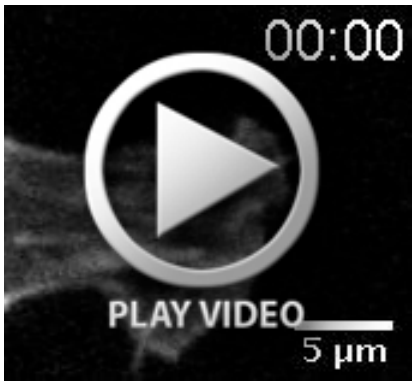
Movie 8. Cropped region of a control cell expressing CH-Utr-GFP treated with dynasore

Movie 9. Cropped region of a control cell expressing CH-Utr-GFP treated with Dyngo-4a

Movie 10. Cropped region of a dynamin TKO cell expressing CH-Utr-GFP treated with DMSO



Movie 11. Cropped region of a dynamin TKO cell expressing CH-Utr-GFP treated with dynasore



Movie 12. Cropped region of a dynamin TKO cell expressing CH-Utr-GFP treated with Dyngo-4a